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Frozen Section Technique to Evaluate Early Burn Wound Biopsy: A Comparison with the Rapid Section Technique

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The importance of early diagnosis and treatment of burn wound infection has prompted many efforts to use frozen section technique for processing burn wound biopsies, most of which have been unsuccessful. A frozen section technique which facilitates quick, reliable evaluation of biopsies was developed, and has been used in the evaluation of 169 biopsies over a period of 18 months. The frozen section technique takes 30 minutes compared with 4 hours for the rapid section method. Comparison of diagnoses made using both methods for each of the 169 biopsies produced a 96% coincidence. Each discrepant diagnosis was corrected by the rapid technique approximately 3½ hours after the frozen section diagnosis. The frozen section technique is a generally accurate and rapid means of assessing the microbial status of a burn wound and diagnosing invasive infection. Permanent sections produced by rapid section technique should always be examined to confirm the frozen section diagnosis.

Histologic examination of the burn wound biopsy establishes the diagnosis of burn wound infection (8). Although quantitative cultures have been proposed as a means of identifying burn wound infection, the limitations of culture techniques make it difficult to establish the diagnosis since they do not permit differentiation between colonization and infection (1, 4, 5, 11). Histologic examination of a burn wound biopsy permits the necessary differentiation and is the best method for the detection of burn wound infection. When a rapid section technique is used, about 4 hours are required for preparation of histologic sections. The short processing time required for frozen section has been considered a desirable alternative. In the past, the majority of burn eschars were not suitable for the frozen section method due to the hardness of the eschar, and attempted adaptations of the technique for practical diagnostic use have been unsuccessful. Advances in techniques and equipment which have expanded the usefulness of the frozen section method for bone and cartilage (3, 7, 9, 10) encouraged us to re-evaluate this technique for the early detection of burn wound infection. Rapid section and frozen section methods were compared. The frozen section technique proved successful and appears to be the fastest method to detect burn wound infection.

MATERIALS AND METHODS

Burn wound biopsies were taken from 30 severely burned patients admitted to the United States Army Institute of Surgical Research over an 18-month period. The excisional biopsies measured 1 × 0.5 × 0.5 cm to 2 × 1 × 1 cm and weighed 100 to 500 mg each (Fig. 1). The tissue was received in a fresh state or in saline and processed within 30 minutes after the biopsy was taken. Each biopsy specimen was divided into two portions: one for rapid sectioning and one for frozen sectioning. The rapid sectioning was done by conventional methods. If the subcutaneous fat and dermal collagen were excessive for a single frozen section procedure they were divided at their junction and embedded separately. The tissue was embedded in Tissue TEK II O.C.T. Compound (Ames Company, Elkhart, IN) on pellets in the cryostat and cut with a Cryo-cut II Microtome Model 851C (American Optical, Buffalo, NY). A temperature of −20°C was satisfactory for freezing both dermal collagen and subcutaneous fat. The specimens were sectioned at 10 μ or less. Albumin-coated slides were used to prevent loss of the tissue from the slides during the staining process. The tissue sections on the slide were fixed in 80% ethyl alcohol for 3 minutes and stained. The processing times for the standard Gram's and McManus Periodic Acid Schiff stain procedures are excessive for the frozen section technique and were shortened (2, 6; Table I). Most stains can be used in frozen sections but the time-modified Brown Hopps tissue Gram's stain was the most desirable screening stain and was adequate for detection of both bacteria and fungi. Periodic Acid Schiff and hemotoxylin and eosin stains can also be used for frozen sections.

Permanent sectioning was done either by rapid section or by the routine surgical sectioning technique. Hemotoxylin-eosin

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FIG. 1. Typical burn wound biopsy showing approximate size in centimeters.

TABLE I
Time-modified stains for frozen sections

Time-modified Brown Hopps Tissue Gram's Stain	Time	Time-modified McManus PAS	Time
Fix in 80% ethyl alcohol	3 minutes	Fix 80% alcohol	3 minutes
Rinse in distilled H_2O	10 seconds	Rinse in distilled H_2O	10 minutes
Crystal violet	30 seconds	Periodic acid	1 minute
Rinse in distilled H_2O	10 seconds	Rinse in distilled H_2O	10 seconds
Mordant with Gram's iodine	1 minute	Schiff's reagent	3 minutes
Rinse in distilled H_2O	10 seconds	Wash in running tap water	5 minutes
Gallago's	1 minute	Harris' hemotoxylin	30 seconds
Rinse and blot	10 seconds	Rinse in tap water	10 seconds
Tartrazine and blot	1 minute	1% (HCl) acid alcohol	5 seconds
Cellosolve	10 seconds	Rinse in H_2O	10 seconds
Cellosolve	10 seconds	Ammonia H_2O	2 seconds
Two changes xylene	10 seconds	Dehydrate in graded alcohol 95–100%	10 seconds
Mount in permamount	10 seconds	Two changes xylene	10 seconds
		Mount in permamount	10 seconds

TABLE II
Average times needed for rapid and frozen section preparation

Procedures \ Methods	Rapid Section	Frozen Section
Fixation	Formaline 20 minutes	—
Ultra-Technicon	2 hours	—
Cryostat	—	5 minutes
Embedding	30 minutes	5 minutes
Cutting	30 minutes	5 minutes
Staining	40 minutes	15 minutes
Total Times	4 hours	30 minutes

stain, Brown Hopps tissue Gram's stain and McManus PAS stains were considered the most effective. The required times for staining of frozen and rapid sections are compared in Table II. Histologic evaluation depended upon clear visualization of microorganisms and the depth of penetration of the organisms into the viable tissue. The presence of microorganisms in viable tissue constituted burn wound infection.

RESULTS

Either frozen or rapid section techniques can be used for the expeditious preparation of burn wound biopsy specimens for histologic examination (Fig. 2). The frozen section technique requires only 30 minutes; the rapid section technique requires almost 4 hours. Both techniques appear to be accurate in identifying burn wound infection as indicated by concordance of diagnosis in 162 of 169 (95.8%) biopsies from which sections were prepared by both methods. Thirty-nine biopsies were positive for infection by both techniques. Six biopsies were positive by rapid section technique and negative by frozen section technique and one was positive by frozen section technique and negative by rapid section technique (Table III).

DISCUSSION

In the interpretation of biopsy slides pathologists should be familiar with possible artifacts such as stain precipitate, precipitation of silver from topical creams, and lysosomal granules, all of which may be confused with microorganisms, e.g., Gram-positive or Gram-negative cocci. Stain precipitates and the elastic tissue of dermis may be confused with fungal hyphae (Fig. 3).

Application of the frozen section technique to burn wound biopsies requires several preconditions for success (Table IV). Immediately after harvest biopsy specimens should be transported in the fresh state or in a container filled with saline. Chemical fixation of the tissue before freezing is absolutely contraindicated. Mechanical injury such as folding and crushing of the tissue section during preparation of the frozen section can be prevented by using a sharp microtome knife and by separating the dermal tissue from the subcutaneous fatty tissue. One of the time-modified staining methods should be used to prevent deterioration of the tissue and reduce the risk of separation of the tissue from the slide during the washing procedure.

A falsely negative reading of frozen sections may result from inadequate tissue dehydration. This may cause hazy cellular detail and reduced tissue stain affinity. Additionally, if the tissue sample contains only a few microorganisms a falsely negative reading can occur due to chance. Inexperience on the part of the pathologist can also produce falsely negative readings. Therefore permanent sections should always be examined to confirm frozen section diagnosis and exclude false negatives.

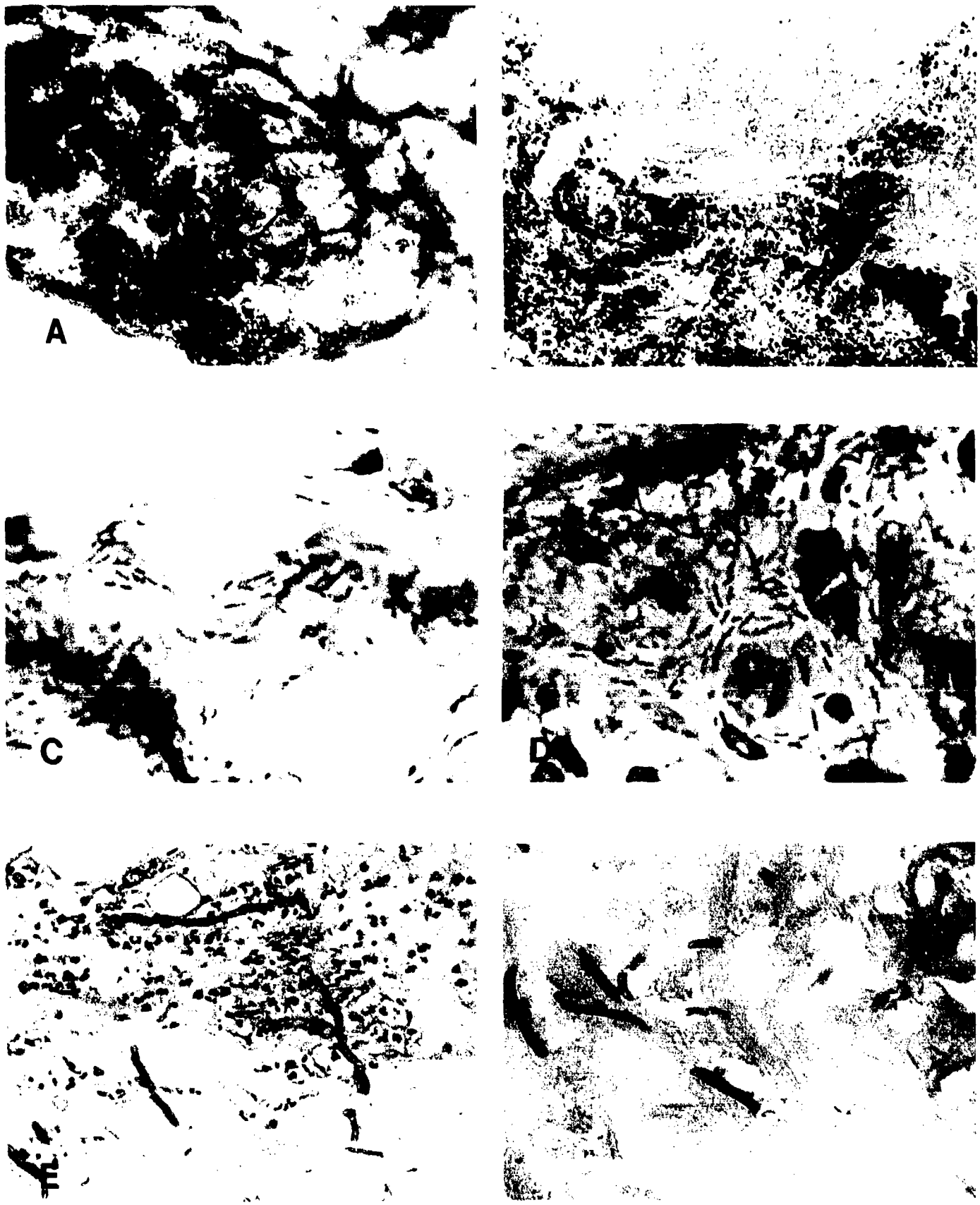

FIG. 2. Frozen section (**A**) and rapid section (**B**) showing *Staphylococcus aureus* in the nonviable dermis, Brown Hopps Gram's Stain 400×. Frozen section (**C**) and rapid section (**D**) showing Pseudomonas organisms in the viable dermis, Brown Hopps Gram's Stain, 400×. Frozen section (**E**) and rapid section (**F**) showing Aspergillus hyphae in the nonviable dermis, PAS 160×.

FIG. 3. Common artifacts found on frozen section that may be confused with microorganisms: A) Lysosomal granules in adipose tissue may be confused with Gram-positive cocci; B) Elastic tissue in dermal collagen may be confused with fungi; C) Stain precipitation artifact may be confused with fungi.

TABLE III
Comparison of the diagnoses between the rapid section method and the frozen section method

		Frozen Section		Totals
		Positive	Negative	
Rapid section	Positive	39	6	45
	Negative	1	123	124
Totals		40	129	169
Coincidence		162/169 (95.8%)		
Discrepancy		7/169 (4.2%)		

TABLE IV
Requirements for successful use of the frozen section method

1) Use of a nonfixative medium for transport.
2) Use high quality mounting media (Tissue-TEK II).
3) Embedding of dermal and subcutaneous tissue separately.
4) Use of optimal cutting temperatures (collagen—13°C; fat—25°C).
5) Use of a modern cryostat.
6) Use of albuminized slides.
7) Use of the time modified Brown Hopps Gram's stain.
8) Preparation of permanent sections for confirmation.

CONCLUSIONS

The frozen section method of evaluating the burn wound biopsy is easy to accomplish and the quality of the slides is comparable to that of the routine rapid section method. The slight decrease in accuracy of frozen section diagnoses as compared to those with rapid section is outweighed by the rapidity with which the diagnosis of burn wound infection can be made and necessary therapy initiated.

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